

CLAIMS

1. An insecticidal protein comprising the sequence:

X<sub>1</sub>X<sub>2</sub>ICTPAGVKCPAALPCCPGLRCIGGVNNKVCR (SEQ ID No. 1).

5 wherein X<sub>1</sub> and X<sub>2</sub> are any amino acid.

- 10 2. An insecticidal protein according to claim 1 wherein X<sub>1</sub> and X<sub>2</sub> are selected from the group consisting of: Glycine; Lysine; Serine; Tyrosine; Alanine; Methionine; Threonine; Glutamic acid; Aspartic acid; Asparagine and Valine.

- 15 3. An insecticidal protein according to claim 2 comprising the sequence:

GKICTPAGVKCPAALPCCPGLRCIGGVNNKVCR (SEQ ID No. 2).

- 20 4. An insecticidal protein having at least 55% identity to a protein according to any of claims 1 to 3.

- 25 5. An insecticidal protein having at least 70% identity to a protein according to any of claims 1 to 3.

- 20 6. An insecticidal protein according to any of claims 1 to 5 wherein the amino acid at position X<sub>1</sub> is modified.

- 25 7. An insecticidal protein according to claim 6 wherein the amino acid at position X<sub>1</sub> is acetylated.

8. An insecticidal protein according to claim 6 or 7 wherein the amino acid at position X<sub>1</sub> is at the N-terminus.

- 30 9. A polynucleotide encoding a protein according to any one of claims 1 to 5.

10. A polynucleotide sequence which is the complement of one which hybridises to a polynucleotide according to claim 9 at a temperature of about 65°C in a solution

- 31 -

containing 6 x SSC, 0.01% SDS and 0.25% skimmed milk powder, followed by rinsing at the same temperature in a solution containing 0.2 x SSC and 0.1% SDS wherein said polynucleotide sequence still encodes an insecticidal protein.

- 5 11. A polynucleotide sequence according to claim 10 comprising the sequence depicted as SEQ ID Nos. 4 to 14.

*JMC BY 10*  
12. An insecticidal synergistic combination comprising a first protein according to any one of claims 1 to 8 and at least one further protein.

13. A combination according to claim 12 wherein said further protein is an insecticidal CRY protein.

14. A combination according to claim 13 wherein the said further protein comprises a sequence selected from the group consisting of SEQ ID Nos. 54 to 59.

*JMC BY 15*  
15. A polynucleotide which comprises regions encoding the first and further protein according to any one of claims 12 to 14.

- 20 16. A polynucleotide according to claim 15 wherein the region encoding said first protein comprises a sequence selected from the group depicted as SEQ ID Nos. 4 to 14.

17. A method of evolving a polynucleotide which encodes a protein having insecticidal properties comprising:

25 (a) providing a population of variants of said polynucleotide and further polynucleotides which encode further proteins, where at least one of said

*JMC BY 7*  
polynucleotides is in cell free form; and

(b) shuffling said variants and further polynucleotides to form recombinant polynucleotides; and

(c) selecting or screening for recombinant polynucleotides which have evolved towards encoding a protein having the said insecticidal properties; and

30

- 32 -

*Mr C. G. Smith*

(d) repeating steps (b) and (c) with the recombinant polynucleotides according to step (c) until an evolved polynucleotide which encodes a protein having insecticidal properties has been acquired wherein said population of variants in part (a) contains at least a polynucleotide according to any one of claims 9 to 11, 15 or 16.

5

18. A method according to claim 17 wherein said population of variants in part (a) contains at least a polynucleotide encoding the protein depicted as SEQ ID No. 1 to 3 and/or said further polynucleotides in part (a) encode a CRY protein.

*Mr C. G. Smith*

A polynucleotide obtainable or obtained by the method according to claim 17 or 18.

20. A protein encoded by a polynucleotide according to claim 19.

21. A DNA construct comprising in sequence a plant operable promoter operably linked to a polynucleotide according to any one of claims 9 to 11, 15, 16 or 19 or a polynucleotide encoding a protein according to claim 20 operably linked to a transcription termination region.

- 20 22. A DNA construct according to claim 21 which further comprises a region which provides for the targeting of the protein product to a particular location.

23. A DNA construct according to claim 21 or 22 which further comprises a region which provides for the production of a protein which acts as a selectable marker.

- 25 24. A DNA construct according to any one of claims 21 to 23 wherein the plant operable promoter is selected from the group consisting of *Agrobacterium rhizogenes* Rld; potato protease inhibitor II; CaMV35S; FMV35S; NOS; OCS; Patatin; E9; alcA/alcR switch; GST switch; RMS switch; oleosin; ribulose bisphosphate carboxylase-oxygenase small sub-unit promoter and other root specific promoters including MR7 promoter (maize); Gos9 (rice) and GOS2 promoters.

30

- 33 -

25. A method of providing a plant or plant part with an insecticidal protein or an insecticidal synergistic combination comprising:
- (a) inserting into the genome of plant material a polynucleotide according to any one of claims 9 to 11, 15, 16 or 19 or a polynucleotide encoding a protein according to claim 20 or a DNA construct according to any one of claims 21 to 24; or
- 5 (a) inserting into the genome of plant material which is capable of producing a further protein, a polynucleotide according to any one of claims 9 to 11, 19 or a polynucleotide encoding a protein according to claim 20 or a DNA construct according to any one of claims 21 to 24; or
- 10 (a) inserting into the genome of plant material which is capable of producing a protein according to any one of claims 1 to 8 or a protein provided for by a polynucleotide according to any one of claims 9 to 11 or 19, a polynucleotide which provides for a further protein; and
- (b) regenerating plants or plant parts from said material; and
- (c) selecting the plants or plant parts having said protein or combination.
- 15 A1
26. A method of providing a plant with a combination according to any one of claims 12 to 14 comprising crossing a first plant which is capable of providing a first protein according to any one of claims 1 to 8 or a first protein provided for by a polynucleotide according to any one of claims 9 to 11 or 19 with a second plant which is capable of producing a further protein and selecting the resultant plant which is capable of producing said combination.
- 20
27. Plants of plant parts obtained according to the method of claim 25 or plants obtained according to the method of claim 26.
- 25
28. Plants or plant parts according to claim 27 wherein said protein or the first protein of said combination is post translationally modified.
29. Plants or plant parts according to claim 28 wherein said protein or the first protein of said combination is acetylated.
- 30

- 34 -

30. Plants or plant parts according to claim 28 or 29 wherein said protein or the first protein is modified/acetylated at the N-terminus.
31. Plants or plant parts according to claim 27 to 30 selected from the group consisting of melons, mangoes, soybean, cotton, tobacco, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tomato, alfalfa, lettuce, maize, wheat, sorghum, rye, bananas, barley, oat, turf grass, forage grass, sugar cane, pea, field bean, rice, pine, poplar, apple, peaches, grape, strawberries, carrot, lettuce, cabbage, onion, citrus, cereal, nut plants or other horticultural crops.
- 10 32. A method of providing a plant or plant part with a further desired agronomic trait comprising:
- (a) inserting into the genome of plant material a polynucleotide which provides for the desired agronomic trait; and
- (b) regenerating plants or plant parts from said material; and
- (c) selecting the plants or plant parts having said desired agronomic trait wherein said plant material is capable of producing an insecticidal protein according to any one of claims 1 to 8 or a combination according to claims 12 to 14; or crossing a first plant according to any one of claims 27 to 31 with a second plant which provides for said further desired agronomic trait and selecting the resultant plant which is capable of producing the further agronomic trait.
- 20 33. A method according to claim 32 wherein the further desired agronomic trait is selected from the group consisting of: herbicide resistance; insect resistance; nematode resistance; stress tolerance; altered yield; altered nutritional value or any other desirable agronomic trait.
- 25 34. Plants or plant parts obtained according to the method of claims 32 or 33.
- 30 35. An insecticidal protein comprising the sequence depicted as:  
-X<sub>1</sub>-X<sub>2</sub>-X<sub>3</sub>-Cys<sub>4</sub>-X<sub>5</sub>-X<sub>6</sub>-X<sub>7</sub>-X<sub>8</sub>-X<sub>9</sub>-X<sub>10</sub>-Cys<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-X<sub>14</sub>-X<sub>15</sub>-X<sub>16</sub>-Cys<sub>17</sub>-Cys<sub>18</sub>-X<sub>19</sub>-X<sub>20</sub>-X<sub>21</sub>-X<sub>22</sub>-Cys<sub>23</sub>-X<sub>24</sub>-X<sub>25</sub>-X<sub>26</sub>-X<sub>27</sub>-X<sub>28</sub>-X<sub>29</sub>-X<sub>30</sub>-X<sub>31</sub>-Cys<sub>32</sub>-X<sub>33</sub>- (SEQ ID No. 60)

- 35 -

wherein X<sub>1-3</sub>, 5-10, 12-16, 19-22, 24-31 and 33 is any amino acid.

36. An insecticidal protein having a FASTA opt score greater than 109 when compared with SEQ ID No. 1 using FASTA algorithm Version 3.

5

37. An insecticidal protein obtainable from *Paecilomyces sp.*

- 10 38. An insecticidal protein according to claim 37 obtainable from *Paecilomyces farinosus*.

- 15 39. A method of controlling insects comprising providing at a locus where the insects feed a protein according to any one of claims 1 to 8, 20, 35 to 38 or a combination according to any one of claims 12 to 14.

40. Use of a polynucleotide according to any one of claims 9 to 11, 15, 16 or 19 or a DNA construct according to any one of claims 21 to 24 in the production of plants or plant parts which are resistant to insects.

- 20 41. Use of a protein according to any one of claims 1 to 8, 20, 35 to 38 or a combination according to any one of claims 12 to 14 as an active ingredient of a pesticide.

42. Use of a *Paecilomyces Sp.* in the preparation of a pesticide containing as an active ingredient a protein according to any one of claims 1 to 8.

- 25 43. Use according to claim 42 wherein said *Paecilomyces Sp.* has been modified to allow for increased production of a protein according to any one of claims 1 to 8.

- 30 44. A recombinant micro-organism which provides for production of a protein according to any one of claims 1 to 8, 20, 35 to 38 or a combination according to any one of claims 12 to 14 or a protein encoded by a polynucleotide according to any one of claims 9 to 11, 15, 16 or 19.

- 36 -

- 5 *M G 10 Cn R*
45. A recombinant baculovirus which comprises a protein according to any one of claims 1 to 8, 20, 35 to 38 or a combination according to any one of claims 12 to 14 or a protein encoded by a polynucleotide according to any one of claims 9 to 11, 15, 16 or 19.
- 10 46. Use of a baculovirus according to claim 45 in a method of controlling insects.
47. An insecticidal protein which is capable of reacting with a monoclonal antibody raised to the protein depicted as SEQ ID No. 1.
- 15 48. A composition comprising an insecticidally effective amount of a protein according to any one of claims 1 to 8, 20, 35 to 38 or a combination according to any one of claims 12 to 14 or a protein encoded by a polynucleotide according to any one of claims 9 to 11, 15, 16 or 19 and optionally an agriculturally acceptable carrier and/or a diluent and/or an insect attractant.
- 20 *M G 11 Z*
49. A polynucleotide comprising a first region encoding an insecticidal protein according to any one of claims 1 to 8, 20, 35 to 38 and a further region encoding a further protein.
50. A plant cell comprising a protein according to any one of claims 1 to 8, 20, 35 to 38 or a combination according to any one of claims 12 to 14 or a polynucleotide according to any one of claims 9 to 11, 15, 16 or 19.
- 25 51. An insecticidal protein comprising the motif depicted as -LPCCPG- (SEQ ID No. 63).